Commentary

Growth Factors in the Nucleolus?

Thoru Pederson

Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01605

In eukaryotic cells containing tandem repeated ribosomal RNA genes, there appears a specialized region of chromatin, carrying out gene transcription, rRNA processing, and nascent ribosomal subunit assembly—the nucleolus. One of the first intracellular structures to be described (Montgomery, 1898; Franke, 1988), the nucleolus was established in the 1960s as the center of ribosome synthesis (Schultz, 1966), culminating in the first visualization of genes in action (Miller and Beatty, 1969). Recently it has become evident, however, that the nucleolus is also the site of several nonribosomal RNA processing and assembly functions. These include maturation of signal recognition particle RNA (Jacobson and Pederson, 1998), transfer RNA, U6 small nuclear RNA, telomerase RNA, and some mRNAs (Pederson, 1998). In the envisioned proto-eukaryotic ancestor, various RNA processing and ribonucleoprotein assembly events may have all initially been centered around a minimal genome to chemically facilitate the production of essential gene readout machines. The nucleolus of today's eukaryotes may be a descendent of this concentrated region of genome readout. Beyond these recently discovered, diverse nonribosomal RNA processing and ribonucleoprotein assembly events in the nucleolus, there is another set of provocative findings: the presence in nucleoli of mitogenic growth factors as well as cell cycle and growth factor-related proteins.

Growth Factors and Growth Regulatory Proteins in the Nucleolus

Although there are numerous studies (of varying quality and cogency) indicating the presence in nuclei of polypeptide growth factors and, in a few instances, growth factor receptors (Burwen and Jones, 1987; Jans, 1994; Stachowiak et al., 1997), the more salient point for this commentary is that during the past few years a number of mitogenic growth factors and other growth regulatory proteins have been observed to be localized in the nucleolus. These include basic fibroblast growth factor (Bouche et al., 1987; Baldin et al., 1990; Moroianu and Riordan, 1994), acidic fibroblast growth factor (Moroianu and Riordan, 1994), angiogenin (Moroianu and Riordan, 1994), parathyroid hormone-related peptide (pTHrP)[1] (Henderson et al., 1995; Nguyen and Karaplis, 1998), the Werner syndrome (WRN) gene product (Marciniak et al., 1998), the B-type cyclin $p63^{cdc13}$ (Gallagher et al., 1993), and the oncogene c-*myb*–associated protein p160 (Tavner et al., 1998). Although this list seems brief at first glance, it is actually rather impressive when one considers that most surveyors of the nucleolar protein landscape have had no particular motivation to look for growth factors altogether.

Several of these aforementioned studies involved incubating cells with exogenous growth factors, ostensibly approximating the in vivo paracrine/autocrine situation. However, the cited study of pTHrP involved its transient expression and nucleolar localization (Henderson et al., 1995) and, in addition, these investigators observed that endogenous pTHrP was present in nucleoli of cultured neonatal osteoblasts. There are precedents in which alternative mRNA splicing or a switch in initiation codon leads to the production of a growth factor lacking its normal secretory signal peptide and, in some instances, subsequent nuclear localization (Maher et al., 1989; Acland et al., 1990; Quarto et al., 1991; Vagner et al., 1996). The case of pTHrP illustrates that this "intracrine" pathway can lead not only to nuclear translocation but also localization in the nucleolus.

Tyrosine Phosphorylation, a Hallmark of Growth Factor Signal Transduction, Also Occurs in the Nucleolus As Does Other Signal Transduction-related Protein Phosphorylation

Among numerous reports of protein tyrosine phosphorylation/dephosphorylation reactions in the nucleus altogether (David et al., 1993; McLaughlin and Dixon, 1993; Rohan et al., 1993), there are two reports of tyrosine phosphorylation of specifically nucleolar proteins: the yeast immunophilin Fpr3 (Wilson et al., 1997), and the protozoan *Trypanosoma brucei* proteins Nopp44/46 (Das et al., 1996). The tyrosine phosphorylation of Fpr3 is mediated by casein kinase II (Wilson et al., 1997), an enzyme that usually phosphorylates only serine or threonine in higher eukaryotes but which also has specificity for tyrosine in

Address all correspondence to T. Pederson, Department of Biochemistry and Molecular Biology, University of Massachusetts Medical School, Worcester, MA 01605. Tel.: (508) 856-8667. Fax: (508) 856-8668. E-mail: thoru@sci.wfbr.edu

1. *Abbreviation used in this paper*: pTHRP, parathyroid hormone-related peptide.

both *Saccharomyces cerevisiae* and *Saccharomyces pombe*. The immunophilin family of which the yeast nucleolar Fpr3 protein is a member is defined by the immunosuppressive drug FK506 and its numerous intracellular targets (Sigel and Dumont, 1992), and it is noteworthy in the present context that one of these targets, the protein FKB25, is known to associate both with casein kinase II and the nucleolar protein nucleolin (Jin and Burakoff, 1993).

Of course, two mere instances of tyrosine phosphorylation of nucleolar proteins do not, by themselves, provide anything more than a zephyr of a connection to the nucleolus as a center of mitogenic growth factor action, although the Fpr3-FKB25–nucleolin connection is certainly provocative. In addition to tyrosine phosphorylation of nucleolar proteins, it has been observed that basic fibroblast growth factor binds to the β subunit of casein kinase II, stimulating the serine/threonine phosphorylation of nucleolin (Bonnet et al., 1996). Moreover, the nuclear envelope phosphoinositide-based protein phosphorylation system (Martelli et al., 1992; Divecha et al., 1993; Raben and Jaken, 1994) displays multiple isoforms of protein kinase C (PKC), some of which are nucleolar (Beckmann et al., 1994). Indeed, one of the targets of nerve growth factor-triggered PKC phosphorylation is, once again, nucleolin (Zhou et al., 1997), and in a recent investigation evidence was reported that neomycin blocks nuclear translocation of angiogenin by virtue of this antibiotic's inhibitory action on phospholipase C (Hu, 1998).

An intriguing observation in relation to signal transduction-related phosphorylation of nucleolar proteins is the finding that the aforementioned yeast nucleolar FK506 binding protein has homology with a protozoan (*Naegleria gruberi*) nucleolar protein, BN46/51 (Goeckeler et al., 1997), which is also found in the *Naegleria* basal body (Trimbur and Walsh, 1992). The basal body is a specialized centriole that nucleates the assembly of flagellar and ciliary microtubules into the 9 + 2 motif of the axoneme. (*Naegleria* shifts from ameboid locomotion to flagellar-propelled swimming during its life cycle.) The basal body is an autonomously replicating organelle, in which the presence of a nucleic acid component has been long sought unsuccessfully. That the nucleolus and basal body of *Naegleria* share a common protein is itself interesting with regard to the evolution of these two organelles, and the fact that this protein shares homology with the yeast nucleolar FK5060-binding protein serves to raise the possibility of a connection to signal transduction pathways.

Perspective

There is rarely anything to lose from thinking in the context of evolution. The typical growth factors we know today arose, or were refined, with the advent of metazoan life, evolving (probably from ancient chemoattractants) as ligands in step with their coemerging cell surface receptors. However, some of today's secreted growth factors might be evolutionarily descended from proteins that were once capable of triggering cell division from within, and indeed, as mentioned above, such intracrine pathways appear to operate in extant eukaryotes. In the single-cell forebears of metazoa, it is likely that intracellular signal proteins would have already been hewn to target on genomic sites at which growth-promoting genes resided. Such intracellular signaling in the predecessors of the metazoa may have embraced protein tyrosine phosphorylation and may have also shared elements with other replicating domains of the cell in addition to the nuclear genome, the basal body for example. Today, more than two billion years later (Han and Runnegar, 1992), we properly see growth factors as primarily operatives on the cell surface, binding receptors in the intercellular signaling lifestyle that defines metazoan life, notwithstanding concurrently operating intracrine pathways as well.

How do nucleolus-localized growth factors actually work? Of course, all roads that lead to the nucleolus would logically suggest ribosome synthesis as the obvious target of regulation. For example, fibroblast growth factor-mediated phosphorylation of nucleolin has been implicated in turning on ribosome production (Bouche et al., 1987; Bonnet et al., 1996). It is certainly true that the rate of rRNA gene transcription, pre-rRNA processing, and nascent ribosome subunit assembly—the canonical roles of the nucleolus—could be downstream elements in positively controlling cell growth, although the typically long half-life of cytoplasmic ribosomes (Loeb et al., 1965) might often emerge as a limiting parameter in negative growth control loops. But the recent reports that other, nonribosomal RNA maturation events also occur in the nucleolus (Jacobson and Pederson, 1998; Pederson, 1998) now suggest other targets of growth control therein. The presence of growth factors and cell cycle-related proteins in the nucleolus currently constitutes something of an intellectual way station, neither an established piece of orthodoxy on the one hand nor necessarily an opaque box on the other. We need to know how these nucleolus-localized growth factors operate in gene readout, as the era of the plurifunctional nucleolus now comes into view.

J. Darnell (Rockefeller University, New York, NY), A. Martelli (University of Trieste, Trieste, Italy) A. Pardee (Dana Farber Cancer Institute, Boston, MA), J. Riordan (Harvard Medical School, Boston, MA), J. Wang (Michigan State University, East Lansing, MI), and E. Wieben (Mayo Clinic, Rochester, MN) are thanked for their helpful comments on a draft of the manuscript. The author is grateful to W. Franke for his critical advice on the original discovery of the nucleolus.

This work is supported by a grant from the National Institutes of Health (GM-21595-22).

Received for publication 10 August 1998 and in revised form 16 September 1998.

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